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(54) Title: DIAGNOSIS OF GASTRIC AND LUNG DISORDERS (57) Abstract For diagnosis or monitoring of gastric or lung conditions, particularly <u>H.pylori</u> infection or tuberculosis, a gas sample is passed to a multiplicity of chemical sensors which generate electrical outputs. These outputs are passed to a data processing system, preferably a hybrid intelligent system employing a search optimisation engine of genetic algorithms and a multiplicity of neural networks. This determines distinctive patterns characteristic of particular disease states.		

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DIAGNOSIS OF GASTRIC AND LUNG DISORDERSTechnical Field

5 This invention relates to a system for use in
diagnosing and/or monitoring gastric and lung disorders
by analysing gas samples. These may be sampled from
patient directly (e.g. gas sample from stomach or breath)
or generated from a sample affected by a said disorder
(e.g. sputum) e.g. via enzyme treatment.

10 *H. pylori* (HP), infection is known as the most
common gastrointestinal bacterial disease world-wide. It
is now accepted as the major cause of gastroduodenal
ulceration in over 80-90% of patients, and chronic
atrophic (type B) gastritis (1). It has been also
15 recognised as type 1 human carcinogen and suggested as a
co-factor in the development of gastric adenocarcinoma,
and mucosa-associated lymphoid tissue (MALT) lymphoma
(2). Two patterns of HP infection have been recognised:
a. in developing countries where a large proportion of
20 children are infected and almost all adults in different
age groups have a chronic HP infection, and b. in western
developed countries, where the prevalence of infection
increases from age 20 onwards (3).

Current tests used to detect HP are either invasive
25 (endoscopy, biopsy, histology, culture, rapid urease
test, Polymerase Chain Reaction) or non-invasive
(serology, ¹³C urea breath test). All the above tests vary
in their sensitivity and specificity and the choice of
test will depend whether the aim is to detect infection
30 or to test the success of eradication treatment.

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Unfortunately, despite the introduction of new serological and molecular biological techniques, contamination effects, high cost and the need for high skilled personnel can severely limit their diagnostic efficiency. Most of the above techniques are not able individually to: a. Characterise and discriminate between different gastric disease stages, focusing only on HP detection and not recognition of disease level, b. offer a complete diagnosis in the form of a rapid bedside technique (for ¹³C-urea breath test it generally takes more than 48hrs to deliver results due to the lack of a mass spectrometer in the endoscopy unit, and the need for additional personnel to perform the test), c. offer an easy-to-use and inexpensive test (PCR and serology need additional skilled personnel, and contamination effects lead to the need to repeat tests, urea-labelled pill is expensive to produce, histology and culture require highly skilled personnel) (4,5), d. facilitate modern pattern recognition methods and artificial intelligence to store, analyse and predict patient data, e. provide individually a single "gold standard" technique and to encompass several aspects of gastric disease. There is always a need to combine several diagnostic tests like the rapid urease test, endoscopy, culture, histology and PCR in order to detect infection and characterise a certain disease stage. However in most UK hospitals, endoscopy and culture remain the standard technique.

Tuberculosis (TB) is caused by Mycobacterium tuberculosis (MTB) and is a major public health problem in many countries world-wide with particular significance

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in developing countries. Approximately one third of the world population (1.9 billion) is infected with MTB. Globally about 10 million new cases of TB are detected every year and 3 million deaths occur annually due to the disease (6). It is also estimated that 16 million people are currently infected with HIV and one third of them will eventually progress to Tuberculosis. Multidrug resistance, poverty, and AIDS have all contributed to global TB resurgence.

10 Globally bronchoscopy and conventional microbiological methods such as culture and microscopy are still considered the most specific and sensitive diagnostic techniques. In most developing countries detection of TB is based exclusively on time consuming and skilled observation, under the microscope, of stained mycobacteria. New methods based upon nucleic acid amplification, such as PCR, strand displacement amplification (SDA), and serology are considered a step forward, however they are laborious very expensive, and suffer from specimen contamination, and low sensitivity.

20 The introduction of Gas Chromatographic techniques in the early 60's prompted investigation of the diagnostic potential of breath or skin volatiles related to several disorders such as cancer, liver cirrhosis and certain biochemical human tissue states (lipid peroxidation) (7,8,9,10,11). Additionally workers in the former Soviet Union have also reported the existence of volatile fatty acids and other biomarkers in the exhaled breath of TB patients (12), and very recently Wang C-H et al. have detected a significant amount of exhaled nitric

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oxide in active pulmonary TB (13). Although all of the above methods are characterised as laborious, expensive and unable to give reproducible results due to lack of powerful artificial intelligence software or equipment .

5 inefficiency, they showed the existence of abnormal concentrations of endogenous volatile mixtures due to the onset of infection. The diagnostic power of odours has been recognised since the 4th century BC. In recent years several workers have investigated the production of

10 certain odours due to infection and destruction of human tissue (14, 15). The first model of an artificial electronic odour detection system was described during the early 1980's and attempted to mimic some functional characteristics of the human olfactory system. Since

15 then, a significant amount of research has been undertaken to design new integrated sensor array systems and apply them, principally, in the food industry (16). Moreover in the past two years scientists have reported novel applications in microbiology (17), diabetes

20 diagnosis (18), and intrapulmonary volatile pattern discrimination (19). *In vitro* experimental work completed in our facilities has revealed the existence of certain volatile patterns of MTB, HP and other gastroesophageal pathogens.

25 The invention can also be applied to analysis of gas samples generated in vitro from samples obtained from patents, e.g. sputum samples.

Disclosure of Invention

In a first aspect the invention provides apparatus

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for use in diagnosing and/or monitoring gastric and/or lung disorders comprising

(a) a sampling system for collecting a gas sample generated by a patient or generated from a sample taken
5 from the patient;

(b) an array of gas sensors each having a different pattern of sensitivities to potential components of the gas sample and being adapted to provide an electrical output signal in response to detection of one or more of
10 said components; and means for passing gas from said gas sample to said array;

(c) a data processing system arranged to receive said electric output signals, said data processing system being adapted to analyse the output signals to detect
15 patterns indicative of the presence of predetermined disorders and/or stages of predetermined disorders.

In a second aspect the invention provides a method of diagnosing and/or monitoring gastric and/or lung disorders comprising

20 (a) collecting a gas sample generated by a patient or generated from a sample taken from the patient;

(b) passing gas from said gas sample to an array of gas sensors each having a different pattern of sensitivities to potential components of the gas sample
25 and being adapted to provide an electrical output signal in response to detection of one or more of said components; and

(c) passing said output signals to a data processing system which analyses output signals to detect patterns
30 indicative of the presence of predetermined disorders

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and/or stages of predetermined disorders.

For generating the gas to be sample we may use a natural odour generation system. For gastric analysis this may involve giving a pill or a solution of non-
5 radioactive and unlabelled urea (10 times cheaper than labelled alternatives and harmless) to the patient suitably 30 minutes before endoscopy after a period, e.g. 16 hrs, of fasting. For lung air analysis, a period, e.g. 16 hrs, of fasting may suffice, optionally with
10 administration of harmless biochemical inducers able to concentrate in the lungs and metabolised by mycobacteria or cancer cells.

A preferred type of embodiment uses a sniffing endoscope comprising:

15 (1) A bag sampling system to collect and equilibrate the intrapulmonary and intragastric volatiles (the latter optionally in the presence of gastric juice), which will be connected directly to one endoscopic channel by using non-toxic, e.g. Teflon, tubing. After each gas sample or
20 "sniff", clean (e.g. carbon filtered) air passes through the channel to avoid contamination from patient to patient. Volatile substances are transferred very rapidly into an inexpensive sampling bag (one bag per patient) by applying a suction pump (suitably 0.7 barr) to avoid
25 losing any volatiles.

(2) An array of gas sensors that interact in a unique way with individual gas molecules or complex odour mixtures and transform physicochemical interaction to electronic signals captured by specific data acquisition
30 software (electronic nose).

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(3) Software and microprocessor for fast odour recognition: A hybrid intelligent system that controls automatically a search optimisation engine of genetic algorithms to identify the best sensor parameters and the most reliable configurations of a group of back propagation neural networks (NN) which finally learn volatile profiles and identify disease patterns.

Expert systems employ a type of hybrid information processing which is now replicated in a new generation of adaptive machines. At the heart of these adaptive machines are intelligent computing systems some of which are inspired by natural mechanisms. NNS can learn to recognise patterns by repeated exposure to many different examples. They are good at recognising complex patterns, from financial training to medical imaging. Genetic algorithms are also naturally inspired and based on the biological principle of "survival of the fittest". A genetic supervisor evolves a problem's solution over many generations, with each generation having better solution than its predecessor. Both techniques are good at explaining their decisions but they cannot automatically acquire the rules they use to make those decisions. These limitations favour the use of hybrid intelligent systems. Like Kobe Steel Plant Japan, which uses a hybrid of different intelligent techniques to solve sub-tasks of the problem (21).

The "sniffing" endoscope offers the following advantages:

Fast and inexpensive collection of volatile samples, which are representative of the actual physical and

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biochemical status of the human lung and the stomach.

Recognition and discrimination of different disease stages in the stomach and the lung before and after treatment.

5 Storage, and rapid analysis (10 min) of patient data in a bedside diagnostic system.

Considerable reduction in the cost of tests.

Some embodiments of the invention will now be described with reference to the accompanying drawings.

10 Brief description of drawings

Fig 1 is a schematic drawing of a data processing system in which several parallel NNs optimised by a specific genetic algorithm can be trained, tested and run automatically by an expert intelligent system which will
15 be able to apply certain rules extracted from experimental results and laboratory experience (20,21).

Fig 2 is a schematic view of a "sniffing" endoscope which characterises HP atmospheres in artificial stomach by using a hybrid intelligent model: (1A&1B) 2L
20 artificial stomach, (2A&2B) bag sampling system collection of volatiles for odour analysis, (3) 2-way valve polypropylene stoppers, (4) fibre optic endoscope, (5) 3-way valve stopper, (6) gas sensor array and microprocessor unit, (7) activated carbon filter, (8)
25 control sample, 15ml of RO water, (9) Vacuum pump, (10) Data capture software, (11) Hybrid intelligent model comprising genetic algorithm system 12, NN back propagation analysis system 14, and multivariate analysis system 16.

30 Fig 3 : an actual sensor-response curve of a

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H.pylori-enriched media (HPE) headspace. Four parameters have been selected to study the sensor response:

Absorption (Ab: maximum rate of change of resistance),

Desorption (DS: maximum negative rate of change of

5 resistance), Divergence (DIV: maximum step response),

Area (AR: Area under the curve).

Fig 4: graphs showing Twenty-five sample responses "sniffs" characterised by 19 genetically selected sensor parameters. Three graphs can be seen: (N) sterile
10 artificial stomach, (HPN) HP positive article stomach and interaction with certain natural biochemical inducers.

Fig 5: discriminant analysis scores and formation of three separate clusters. An artificial stomach atmosphere containing *H.pylori* and biochemical inducers
15 (HPE) has produced a completely different odour profile. Although sterile artificial stomach (N) and *H.pylori* normal growth (HPN) are closer, still there is a clear distinction between them.

Fig 6: schematic diagram of a flow injection
20 bubbling system that was applied for bacterial odour delivery and detection (WB: water bath, SP: sampling point, SU: sensory unit, CF: carbon filter, F: bio-filter, AF: air flow; CS: control sample.

Fig 7; graphical representation of DA scores
25 between: (av) *M.avium*, (c) control, (p) *P.aeuroginosa*, (tb) Tuberculosis and (sc) *M.scrofulaceum*.

Fig 8: graphical representation of thirty-eight sensor parameters showing a clear discrimination between:
(m) *M.avium* and *M.scrofulaceum*, (p) *P.aeuroginosa* (tb)
30 MTB and (c) control sterile cultures.

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Fig 9: graphical representation of GA-NN prediction confidence ($\lambda \approx 1$) of 10 sputum samples;

Fig 10: graphical representation of non-linear nature and complexity of 46 sputum pulses and 5 groups of patterns;

Fig 11: graphical representation of DA-cv separation and correct classification;

Fig 12: graphical representation similar to Fig 11 for a different experiment.

10 Modes for carrying out the invention

Example 1 "Sniffing" the static headspace of an artificial stomach infected with *H.pylori*

1.1 Odour generating system

Following successful isolation of HP from gastric biopsies and growth in tissue culture flasks (75cl Corning), containing 70ml brain heart infusion broth (Oxoid), 5% serum bovine (Oxoid) and antibiotic supplement (Vancomycin 10mg L⁻¹, Trimethorpin lactate 5mg L⁻¹, Cefsulodin 5mg L⁻¹, Amphotericin B 5mg L⁻¹) (Oxoid), three separate treatments were prepared, adjusted to 10⁷ cells ml⁻¹ in media of pH 7.3 and inoculated into 2L urine drainage bags (inflated with carbon filtered air) (Simpla), each containing an anaerocult C sachet (Merck) to create a microaerophilic atmosphere which constitutes the *in vivo* HP microbiotic environment and favours its metabolic activation: (I) *H.pylori* (HPE) in 80ml of medium containing BHI-5% serum bovine, 5ml of 15% sterile urea solution (Oxoid), 0.75mg ml⁻¹ L-asparagine, and L-glutamine (Sigma). (II) *H.pylori* (HPN), normal growth in 80ml BHI-5% serum bovine with no additives. (III) Sterile

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medium (N) containing 80 ml BHI-5% serum bovine plus urea, glutamine and asparagine as described above. Replicate samples of each treatment were incubated for 100 minutes at 37° C and then placed randomly, one each time, in a 6L plastic container (Gio Style) (1A, 1B: artificial stomach and 2A, 2B: Bag sampling system figure 2).

1.2 Odour delivery system

Following 100 min of incubation each bag was placed in a 6L plastic container and connected via a 2-way valve stopcock (BDH) and Teflon tubing (Tygon) to the anterior end of a fibre optic endoscope (Olympus). The other endoscope's channel end was connected via Teflon tubing and a 3-way stopcock to a bag sampling system (plastic container & 2L sampling bag) which was also connected to a vacuum pump

(10 L min⁻¹, Patterson) (Figure 2). The volatile collection system is based on a continuous open airway channel between the artificial stomach (1A & 1B figure 2), the endoscope biopsy channel (4 figure 2) the sampling bag (2B figure 2) and a vacuum pump (9 figure 2) that is capable of rapid transfer (30 sec) of the intragastric atmosphere to the electronic nose apparatus, for direct odour analysis (6,7,8 figure 2).

1.3 Odour detection system

An electronic nose (Bloodhound Sensors, Leeds, UK), which employed 12 conducting polymer sensors was used. Specific selection and tailoring of polymers, doping materials and precise manufacturing process can make each

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gas sensor consistently responsive to different volatile groups. Physicochemical interaction between the volatiles and the conducting polymer surface produces a change in resistance, which can be amplified and analysed through a data capture software (Bloodhound Sensors, 10 figure 2). The sensory unit employed a control sample container (8, figure 2) that produces two calibration reference points, a baseline and a control sample. Activated carbon-filtered air is passed over the sensor surface and generates the baseline (flow: 4ml min^{-1}) (7, figure 2). The control sample unit contained 15ml of sterile water and was used to confirm that the reference point was not affected by drift. A specific sampling profile used 4 seconds of absorption time and 12 seconds of desorption time.

1.4 Odour recognition system and data analysis

Figure 3 describes a real-time sensory response curve taken from a *H.pylori* volatile headspace. Twelve sensor responses and 4 parameters created a set of 48 normalised input variables. Twenty-five samples were collected: a. 8 from sterile atmosphere (N), 8 from HP (normal growth, HPN) atmosphere and 9 samples from HP (enriched media, HPE) atmosphere of enhanced volatility (Figure 4). The previous data was divided into two groups randomly: a. Training data (5 samples N, 5 samples HPN, 5 samples HPE) 60% of all data, b. Test data "unknown" (3 samples N, 3 samples HPN, 4 samples HPE) 40% of all data. The latter was kept out of Neural Network (NN) training. A novel hybrid intelligent system of Genetic Algorithms-NNs (Neuralyst, Brain Maker, USA) and multivariate

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techniques (XlStat, France) was employed. Genetic training uses a special type of optimisation technology, which consists of models of a selective evolutionary process. This used an evolutionary combination of all the three strings and an addition of a mutation rate, which eventually produces a "phenotype", a new more evolved NN architecture. Evolution towards the most successful NN configuration is processed through successor generations. Each NN structure in the generation is evaluated by the lowest error achieved after a certain number of epochs. The input parameter set is represented by an inclusion rate of 0.77. Furthermore a population size of 4 was set to determine the number of phenotypes evolved in each generation. Immigration pool mode was also used to replace the weakest phenotypes. A set of 1 for cross breeding determines the frequency of intermingling of features on the same string. Finally a mutation rate of 0.6 was applied to evolve new NN structures.

A genetic algorithm-NN back-propagation employing a specific architecture of 19 input neurones (sensor parameters), a learning rate of 0.94878 and a momentum of 0.354654 after 10 generations of neural evolution, achieved a 93% prediction rate. Nine out of 10 "unknown" samples were identified correctly and only HPN3 was confused with (N) sterile artificial stomach (Table 1).

A subset of the genetically input sensor parameters was used to perform Discriminant analysis (DA). DA identified a set of sensor parameters that best discriminated between the three tested classes. For maximum discrimination the following two conditions had

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to be satisfied: a. the distance between the bacterial clusters should be as far as possible and b. distances within each bacterial cluster should be as close as possible. Eventually DA identifies a new axis Z such that a new variable from rough sensor data could provide the maximum discrimination. The above multivariate linear technique showed a complete distinction between HPE and the other two classes. Additionally both HPN and N samples, although being very close, have formed two distinctive clusters (Figure 5).

Example 2. Sniffing" Tuberculosis *in vitro*.

2.1 Clinical isolates

All bacteria were isolated from patients at the chest unit and assigned a Hospital number: *Mycobacterium tuberculosis* (MTB) (RIVM myc 4514), *M. avium* (RIVM myc 3875), *M. scrofulaceum* (RIVM myc 3442) and *Pseudomonas aeruginosa* (AMC 23123).

2.2 Cultural system/Odour-generating model

The above bacterial isolates were cultured in tissue flasks (75cl Corning) containing Tween albumin medium (Oxoid) to a final volume of 70ml until they reached their stationary growth phase (4 weeks for MTB, 2 weeks for the other two mycobacterial (Myc) species and 36 hrs for *P. aeruginosa* (P) at the same time. Conventional diagnostic microbiology and optical density measurements confirmed satisfactory growth of each species. The vent-caps of the bottles were sealed with paraffin film (Nesco) to concentrate the metabolic production of bacterial volatiles (7 days for Myc and 12 hrs P). A number of sterile Tween-albumin samples (C) were also prepared and cultured for 4

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weeks under the same conditions with the pathogenic cultures.

2.3 Odour delivery (Flow injection-bubbling system) and detection (Fig 6)

5 When bacterial cultures reached their stationary growth phase, they were transferred, together with some control replicates in a 37°C water bath and left there for 15 min to equilibrate. Each flask was connected with a specifically designed air-filtered sparging system, which
10 consisted of Teflon tubing, a hydrophobic bio-filter (0.45µm, PTFE Whatman Hepavent) to reduce humidity over the sensor surface and an activated carbon filter to ensure clean air above the bacterial headspace. The sampling point was adjusted to a set height above the culture. A flow rate
15 of 200ml⁻¹ was set automatically by the sensory unit (Figure 6). Additionally, environmental conditions, at the sampling point, in the water bath and in the laboratory were monitored continuously in order to establish a standardised sampling regime. The volatile sensing system was the same
20 as in example 1, except that a tissue culture flask (75cl Corning) containing 70ml of sterile water was used as control sample to prevent a drift at the reference point and a new sampling profile was used of 6 sec of absorption and 14 sec of desorption time.

25 2.4 Odour recognition and data analysis

 Five parameters (AR: area, DV: divergence, DS: desorption, AB: absorption and Ratio: AB/DS) and 14 conductive polymer sensors extracted 70 sensor parameters, that carried 38 input/train-normalise signals and 15
30 "unknown" randomly selected "sniffs". Data processing

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employed a hybrid intelligent model of genetic training, optimisation of back propagation NNs and multivariate techniques. Genetic inclusion rate of 0.88, a mutation rate of 0.6 and a cross breeding of 1 were used together with an immigration pool mode to evolve new NN phenotypes. After 100 generations of neural evolution, a 3-layer back propagation NN (63-26-5) carrying a learning rate of 0.414, a momentum of 0.9262, an input noise of 0.0365 and a testing tolerance of 0.5 achieved a NN prediction rate of 96%. Fourteen out of 15 samples were identified correctly (Table 2). Two subsets of genetically selected input sensor data were used to perform DA. The first subset of normalised data (48 sensor parameters) demonstrated a clear distinction between all bacterial classes (Figure 7). Additionally the second data set (38 sensor parameters) managed to discriminate between (c): control, (tb): MTB, (m): *M. avium* & *M. scrofulaceum* and (p): *P. aereuginosa* (Figure 8).

Example 3. TB-Sense: Human sputum enzymatic treatment enables *Mycobacterial* species volatile pattern recognition.

3.1 Introduction

Analysis of sputum may provide valuable clinical and physiological information.

The identification of *Mycobacterium* sp. in sputum is laborious and time-consuming.

The initial investigation requires staining and microscopic examination of sputum.

It takes a minimum of 30min preparation of each sputum sample followed by microscopic examination. An experienced laboratory technician would normally expect to examine no

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more than 6-10 sputum samples per hour. Furthermore, sputum microscopy is positive in a minority of cases and only when there is a heavy bacterial load and the technique can only identify the presence of *Mycobacterium* as a group and cannot differentiate between different strains. This can only be achieved by culture of sputum, which takes a minimum of two weeks and usually 4-6 weeks to correctly identify the sub-species and provide appropriate antibiotic sensitivities. Recently, the rapid molecular examination of species utilising PCR-based technology has become available, however this is technically demanding, requires significant laboratory resources and clean facilities and is expensive (unit test cost approx. £60).

We are currently investigating the hypothetical existence of 2 non-linear dynamic (chaotic) systems: "a" complex metabolic changes during active Tuberculosis (TB) and pulmonary infection and their expression as volatile pulses generated after enzymatic treatment over the headspace of fresh human sputum and "b" the interaction of chemosensory surfaces and sputum volatile groups and their complex pattern recognition by using a hybrid intelligent system. To understand the complexity of in vivo TB-sputum patterns and according to our hypothesis we developed a model of four interdependent factors (Example 1, 2). However in this example the odour generating mechanism consisted of an enzymatic cocktail treatment of sputum which generated within 5 hrs some unique and species-specific headspace volatile patterns.

Two are the enzymatic target groups here, the complex mycobacterial lipid-cell wall and necrotic tissue

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substances and other TB metabolic products present in sputum. Certain enzymes like lipases can interact with complex biological substances such as long chain fatty acids and create novel flavours and volatile compounds.

5 There have been several applications of flavour enzymatic generation in food technology. However in this study we introduced the idea of enzymatic cocktails instead of single enzyme treatments. The introduced novel diagnostic test introduces a unique biochemical "dialogue" with
10 respiratory pathogens and *Mycobacteria* and TB itself *de profundis*. It also forces the respiratory infection and TB to reveal their active metabolic pulses and express them as non-linear complex patterns generated on the surface of an array of 14 conducting polymer gas sensors.

15 3.21 Materials & Methods

3.2.1 Sputum volatile generation.

Forty-six 5ml sputum samples infected with *M.tuberculosis* (tb:10 samples), *M.avium* (av: 10 samples), *Pseudomonas aeruginosa* (p: 10 samples), mixed infection
20 (tb- p, m: 8 samples) and normal/control sputum (c: 8 samples) have been collected from patients attending the Chest Unit clinic of Amsterdam Medical College and Royal Tropical Institute. From each patient sample 1 ml of sputum was mixed with 2ml of an enzymatic cocktail (in Phosphate
25 Buffer Saline 7.7 pH) containing 1mg of porcine pancreas lipase (Sigma) and 4mg of *Aspergillus niger* lipase (Sigma). Forty-six sputum treatments were incubated for 6 hrs at room temperature (23°C).

3.2.2,3 Volatile delivery and detection systems were the

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same as presented in example 2 except that a new sampling profile of 7sec of absorption time and 21sec of desorption time was set.

3.2.4. Intelligent pattern/pulse recognition.

5 Fifty-six sensor parameters and 46 sputum pulses generated a matrix of 2576 sensor data items. Two data groups have been selected randomly: "a" training data (tb: 8, av: 8, m:6, c: 6, p: 8) 78% of all data, "b" test data of 10 "unknowns" (c: 2, tb: 2, m: 2, p: 2, av: 2)
10 22% of all data. The latter was kept out of Neural Network (NN) training. A hybrid intelligent model of genetic algorithms- neural networks (GA-NNs) and multivariate techniques (discriminant analysis-cross validation: DA-cv). The Genetic supervisor used an
15 evolutionary combination of an inclusion rate of 0.93, a population size of 5 (number of NN phenotypes evolved per generation), an immigration pool mode (to replace the weakest NNs in each generation), a set of 3 cross-
breedings (frequency of intermingling of NN features in
20 the same phenotype) and a 0.743 mutation rate. After 10 generations of evolutionary training, the Genetic Supervisor selected a 4-layer (51 input-13-21 hidden-5 output) back-propagation NN which employed a sigmoid function, an adaptive learning rate, a momentum of 0.174
25 and achieved a prediction rate of 96% (Table 3, below).

 Nine out of 10 samples were identified correctly and only one normal sputum sample (c2) was not classified correctly. However its real output (0.48) was very close to preset test tolerance limit of 0.5 (Fig. 9)

30 By extracting the 51 "genetically" selected sensor

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parameters, 4 groups of complex non-linear patterns have emerged (Fig 10). DA-cv also used that set of "genetically" selected parameters and managed to discriminate between: 1. (av, c, m, p, tb: 2 "unknowns" were recognised correctly by cross validation, Fig 11) and 2. (c,b: *Pseudomonas* patterns as bacterial, mb: for *Mycobacteria* av-tb and m for mixed infection tb-p, where another two "unknowns" were classified correctly, Fig 12).

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Table 1: Actual output performance and architecture of a hybrid genetic algorithm- optimised back propagation NN in discrimination between *H.pylori* in enriched media (HPE), sterile artificial stomach (N) and *H.pylori* normal growth (HPN). A correct identification of nine out of ten "unknown" samples has been achieved (1 for true and 0 for false).

	NEG	HPN	HPE	MF		
N1	1.015524	0.004214	0.001395	TRAIN	N1	NEURAL NETWORK STATISTICS
N2	1.032813	-0.03177	0.00032	TRAIN	N2	0.246791 RMS Error
N3	1.025024	-0.02479	-0.00435	TRAIN	N3	30 Number of Data Items
N5	1.025494	-0.0262	-0.00166	TRAIN	N5	28 Number Right
N7	1.017941	0.000767	-0.00022	TRAIN	N7	2 Number Wrong
HPN1	0.00173	0.998538	-0.00237	TRAIN	HPN1	93% Percent Right
HPN2	-0.01505	1.010791	-0.00018	TRAIN	HPN2	7% Percent Wrong
HPN4	-0.03476	1.037109	-0.00079	TRAIN	HPN4	4903 Training Epochs
HPN5	-0.01002	1.005655	-0.00042	TRAIN	HPN5	
HPN7	-0.01932	1.014484	-0.00045	TRAIN	HPN7	Network Parameters
HPE1	-0.01123	-0.01667	0.997363	TRAIN	HPE1	0.94878 Learning rate
HPE2	0.000723	-0.01448	1.006091	TRAIN	HPE2	0.354654 Momentum
HPE4	0.003375	-0.02288	1.007233	TRAIN	HPE4	0.079867 Input Noise
HPE6	0.00116	-0.01247	1.006293	TRAIN	HPE6	0.03 Training Tolerance
HPE8	0.00022	-0.01908	0.9884	TRAIN	HPE8	0.5 Testing Tolerance
N4	1.018076	-0.01841	0.002704	TEST	N4	Genetic Training Statistics
N6	1.036539	-0.03795	0.005759	TEST	N6	10 Generation Count
N8	1.043051	-0.04376	0.007101	TEST	N8	4 Structure Count
HPN3	1.046207	-0.04601	0.002032	TEST	HPN3	0.023432 Least RMS Error
HPN6	-0.00545	1.000385	-0.00045	TEST	HPN6	5000 Least Epochs
HPN8	0.086258	0.928009	0.009586	TEST	HPN8	NETWORK ARCHITECTURE
HPE3	-0.01005	-0.01411	0.994107	TEST	HPE3	# Layers 3
HPE5	-0.00163	-0.01213	1.000989	TEST	HPE5	# Neurons per Layer
HPE7	0.00069	-0.00676	0.965875	TEST	HPE7	19 INPUT
HPE9	0.000925	0.019791	0.999277	TEST	HPE9	15 HIDDEN
						3 OUTPUT

Table 2: Real output performance of a hybrid genetic algorithm optimized back propagation NN in discrimination between headspace atmospheres created by the following clinical isolates: *P.aeruginosa* (ps), *M.tuberculosis* (tb), *M.avium* (av), *M.scrofulaceum* (sc) and control (no growth) (c). A 96% prediction rate has been achieved and 14 out 15 "unknown" samples have been identified correctly.

PSEUDO	TB	MAVIUM	MSCROF	CONTR	MF	
1.001727295	0.001898193	0.000119019	0.000253296	-0.00286724	TRAIN	ps1
1.003372192	0.000991821	0.004046631	-0.004245	-0.00115662	TRAIN	ps2
1.004547119	-0.00635986	0.005892944	-0.00082092	-0.00115662	TRAIN	ps4
0.993569946	-0.00145874	0.007772827	-0.00840759	-0.00333862	TRAIN	ps15
0.999142456	-0.00508423	0.000421143	0.001428223	-0.00135803	TRAIN	ps17
-0.00672913	1.004278564	-0.01008606	-0.01411438	-0.0169342	TRAIN	tb1
-0.0092804	0.998303223	0.009619141	-0.01089172	0.003677368	TRAIN	tb3
-0.00760193	0.99541626	0.011364746	-0.0085083	0.003375244	TRAIN	tb5
-0.01011963	0.997698975	0.000354004	-0.0144165	-0.00018311	TRAIN	tb7
-0.00531921	1.00045166	-0.00196228	-0.01320801	0.008276367	TRAIN	tb10
-0.01089172	1.005487061	-0.00206299	-0.02002258	-0.00975037	TRAIN	tb14
0.000756836	1.010388184	-0.00323792	-0.01508789	-0.00236511	TRAIN	tb16
0.000588989	0.987963867	0.016030884	-0.00948181	0.009988403	TRAIN	tb17
-0.00558777	0.996994019	0.002502441	-0.00817261	-0.00803833	TRAIN	tb18
-0.05	-0.00847473	1.013543701	-0.00313721	-0.01522217	TRAIN	av3
-0.05	-0.00354004	1.010791016	-0.00501709	0.003643799	TRAIN	av5
0.007269287	0.002368164	0.998806763	-0.02583008	-0.00518494	TRAIN	av7
-0.00404358	0.001159668	0.998739624	0.000253296	-0.00528564	TRAIN	av11
-0.00055237	-0.00172729	1.009884644	-0.00834045	-0.00333862	TRAIN	av13
-0.01220093	-0.00820618	1.008172607	0.005255127	-0.00330505	TRAIN	av15
-0.00209656	-0.01656494	1.019082642	-0.02344666	-0.00867615	TRAIN	av19
-0.0067627	-0.0101532	1.013577271	-0.01149597	0.000253296	TRAIN	av21
-0.00058594	-0.01784058	1.013577271	-0.01988831	0.01321106	TRAIN	av23
0.004180908	-0.00337219	-0.00018311	0.990515137	-0.00155945	TRAIN	sc1
-0.02163391	-0.00921326	0.011364746	0.995248413	-0.00860901	TRAIN	sc3
8.54492E-05	-0.00978394	-0.00256653	0.998370361	-0.00515137	TRAIN	sc5
0.000756836	-0.01505432	0.000387573	0.998269653	0.004986572	TRAIN	sc7
-0.00082092	-0.01025391	1.83105E-05	1.000854492	-0.00484924	TRAIN	sc11
0.002401733	0.001092529	-0.00085449	1.001794434	-0.00562134	TRAIN	sc13
-0.00625916	-0.00058594	0.005758667	0.99944458	-0.00189514	TRAIN	sc15
-0.00350647	-0.00924683	0.003408813	0.997900391	0.003710938	TRAIN	sc17
-0.0076355	-0.00713196	0.010055542	0.991320801	-0.00753479	TRAIN	sc23
-0.00797119	-0.00783691	-0.00162659	-0.05	0.996524048	TRAIN	c1
-0.01841125	-0.00397644	-0.00072021	-0.0118988	0.997061157	TRAIN	c3
-0.0118988	-0.00018311	-0.00508423	-0.00273438	0.992764282	TRAIN	c7
0.003308105	-0.0021637	0.008578491	0.010357666	1.008877563	TRAIN	c9
-0.01048889	-0.00588989	0.000387573	-0.01079102	1.001391602	TRAIN	c14
0.007000732	-0.00746765	1.83105E-05	0.004684448	1.004782104	TRAIN	c17
0.671572876	0.001629639	0.001126099	-0.01008606	0.250881958	TEST	ps6
0.176089478	-0.03177185	0.141009521	0.011129761	-0.02113037	TEST	ps13
0.987191772	0.003677368	0.0015625	0.005892944	0.00508728	TEST	ps19
-0.02324524	0.791174316	0.032345581	-0.01498718	-0.01555786	TEST	tb8
-0.0026001	0.829418945	-0.02039185	-0.02455444	-0.00474854	TEST	tb12
0.052755737	0.874182129	-0.01347656	0.06625061	0.099404907	TEST	tb19
-0.05	0.092736816	0.583822632	0.319229126	0.010726929	TEST	av1
0.008108521	-0.00921326	0.977590942	-0.01807556	-0.00535278	TEST	av9
-0.0046814	-0.03761292	0.791543579	0.130938721	-0.01226807	TEST	av17
-0.00354004	0.049969482	0.011297607	0.938053345	-0.01257019	TEST	sc9
0.065142822	-0.01119385	0.021032715	0.89887085	-0.00045166	TEST	sc19
0.009619141	0.018045044	-0.01324158	0.934957886	-0.00266724	TEST	sc21
-0.04812012	0.049197388	0.188577271	-0.05	0.921583721	TEST	c5
-0.03633728	-0.01344299	-0.00776978	-0.00961609	0.974099731	TEST	c11
-0.02149863	-0.02512512	-0.00266724	0.132315063	1.014147948	TEST	c15

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Table 2 cont.

HYBRID GA-NN STATISTICS**Network Run Statistics**

0.171020064 RMS Error
76 Number of Data Items
72 Number Right
3 Number Wrong
96% Percent Right
4% Percent Wrong
24563 Training Epochs

Network Parameters

0.414 Learning rate
0.9262 Momentum
0.0365 Input Noise
0.02 Training Tolerance
0.5 Testing Tolerance
1 Epochs per Update
0 Epoch Limit
0 Time Limit (Hrs)
0 Error Limit (Increase)

Genetic Training Statistics

100 Generation Count
3 Structure Count
0.00429659 Least RMS Error
0 Least Epochs

NETWORK ARCHITECTURE

Layers 3
Neurons per Layer
63 INPUT
26 HIDDEN
6 OUTPUT

CAPTURED DATA: 53 SAMPLES**TRAIN DATA: 38 samples, 72%****"UNKNOWN": 15 samples, 28%****for each species:**

***P.aeruginosa* : 5 train, 62.5%**
3 test, 37.5%
***M.tuberculosis* : 9 train, 75%**
3 test, 25%
***M.avium* : 9 train, 75%,**
3 test, 25 %
***M.scrofulaceum* : 9 train, 75%**
3 test, 25%
Control: 6 train, 66%
3 test, 33%

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Table 3.

0.00958557	-0.0476501	-0.0411713	1.02965698	-0.05	train	TB 10	Genetic Training Parameters
-0.0162628	-0.008609	-0.0444946	0.98148499	-0.05	train	TB 7	0.935 Inclusion Rate
-0.0097504	-0.0416748	0.0014954	-0.0147858	1.014685059	train	M1	4 Max Layers
-0.0017273	-0.0390228	0.0087799	0.01196899	0.988769531	train	M2	30 L2 Neuron Limit
-0.0038757	-0.0381165	-0.0193512	0.00945129	0.99944458	train	M3	25 L3 Neuron Limit
0.00317383	-0.0383514	-0.0352295	0.0052887	1.004446411	train	M5	0.2 Min Learning Rate
0.02217407	-0.0365387	-0.0280792	-0.05	0.995584106	train	M8	0.2 Max Momentum
-0.0361023	-0.0434204	0.0068329	-0.0469116	1.031167603	train	M9	0.03 Max Input Noise
0.831497	-0.0010559	-0.0338531	-0.0256622	0.036776733	test	AV 7	5 Population Size
0.999545	0.2789795	0.0997864	-0.0499329	-0.05	test	AV10	Immigrate Population Mode
0.072662	0.4834875	0.1062042	0.17498779	-0.04771729	test	C2	3 Crossovers
0.09753723	0.596948	0.0615845	-0.0469452	-0.03472595	test	C5	0.743 Mutation Rate
0.46864624	-0.0497986	0.53743	-0.0338867	-0.04684448	test	PS2	# Neurons per Layer
-0.0466095	0.100827	1.04473	-0.0439575	-0.05	test	PS7	51
0.12835388	-0.0485229	0.1013977	0.533771	-0.05	test	TB2	13
-0.0043457	0.0256317	-0.011496	0.959363	-0.02304382	test	TB4	21
0.001328	-0.04258	-0.03355	-0.02956	1.0343903	test	M4	5
-0.017572	-0.0336517	0.062793	0.07638855	0.91203	test	M6	

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Table 3 cont.

Table 3. GA-NN statistics.

M. avium	Control	Pseudo.	TB	Mixed	mf		
103979492	-0.0489594	-0.0078369	-0.0459717	-0.0487915	train	AV1	
0.98440552	-0.0042114	0.0035767	-0.0238159	-0.02539368	train	AV2	Neural
0.99524841	0.0106262	-0.0411377	-0.0365051	-0.01955261	train	AV3	Network Run Statistics
0.99605408	0.0129425	-0.0448975	-0.0152222	-0.00018311	train	AV5	0.2187962 RMS Error
0.96879578	0.0105591	-0.0440247	-0.0259979	0.016534424	train	AV6	50 Number of Data Items
0.98799744	0.0121704	-0.0380493	-0.024353	0.020965576	train	AV8	48 Number Right
102492371	-0.033316	0.0155273	-0.0497986	-0.05	train	AV9	2 Number Wrong
100538635	0.0322449	0.0041138	-0.0498993	-0.05	train	AV4	96 % Percent Right
0.04382629	0.9317688	-0.0414398	0.03687744	-0.03096619	train	C1	4 % Percent Wrong
-0.0470795	10126038	-0.0477173	-0.0471802	-0.03506165	train	C3	1440 Training Epochs
-0.0349945	10058563	0.0109619	-0.0380157	-0.04808655	train	C4	
-0.002533	0.9942078	-0.0008545	-0.0377808	-0.04798584	train	C6	Network Parameters
-0.0103546	0.9702057	-0.0453003	0.01455383	-0.04939575	train	C8	0.506918546 Learning rate
0.01965637	0.9922607	0.0107941	0.00804138	0.014956665	train	C9	0.174077578 Momentum
-0.0007202	-0.05	10074341	-0.0254272	-0.00098877	train	PS1	0.002990204 Input Noise
-0.002063	-0.05	0.990918	-0.0185455	0.018817139	train	PS3	0.03 Training Tolerance
-0.0016266	-0.0170013	0.9928314	-0.0202576	-0.04986572	train	PS4	0.5 Testing Tolerance
-0.0235809	-0.0459381	10462067	-0.047818	-0.05	train	PS5	1 Epochs per Update
-0.0353638	-0.0439575	10484558	-0.0479858	-0.05	train	PS6	Enhanced Parameters
-0.0075684	-0.006897	0.9938049	0.00636292	-0.04993286	train	PS8	Sigmoid Function
0.002771	0.0135468	10441925	-0.0498322	-0.05	train	PS9	Adaptive LR
-0.0217682	-0.0458038	0.9913879	-0.0028351	-0.05	train	PS10	
-0.0025665	0.0333191	-0.0175049	0.98551331	-0.03076477	train	TB1	Genetic Training Statistics
-0.0019623	0.0286865	-0.0088776	100078735	-0.03274536	train	TB3	10 Generation Count
-0.0140472	0.01875	0.0250275	101293945	-0.03841858	train	TB5	5 Structure Count
-0.016095	0.0264038	0.0171051	100108948	-0.03942566	train	TB6	0.019599055 Least RMS Error
-0.0069305	0.0340576	-0.0009552	0.9757782	-0.03123474	train	TB8	5000 Least Epochs
0.00847778	-0.0481873	-0.0383179	102636719	-0.05	train	TB9	

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CLAIMS

1. Apparatus for use in diagnosing and/or monitoring gastric and/or lung disorders comprising:
- 5 (a) a sampling system for collecting a gas sample generated by a patient or generated from a sample taken from the patient;
- (b) an array of gas sensors each having a different pattern of sensitivities to potential components of the gas sample and being adapted to provide an
- 10 electrical output signal in response to detection of one or more of said components; and means for passing gas from said gas sample to said array;
- (c) a data processing system arranged to receive
- 15 said electric output signals, said data processing system being adapted to analyse the output signals to detect patterns indicative of the presence of predetermined disorders and/or stages of predetermined disorders.
- 20 2. Apparatus according to claim 1 wherein said data processing system comprises a hybrid intelligent system that controls a search optimisation engine of genetic algorithms and a multiplicity of neural networks arranged to analyse said output signals using predetermined rules
- 25 and thereby to determine a said pattern.
3. Apparatus according to claim 1 or claim 2 wherein the sample system is adapted to collect samples of gas generated in the patient's stomach or lung.

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4. Apparatus according to claim 3 wherein the sampling system comprises an endoscope having a tubular probe adapted to be inserted into a body cavity of a patient.

5 5. Apparatus according to claim 1 wherein the sampling system is adapted to collect samples of gas generated in vitro from samples of material taken from a patient, said sampling system including a vessel defining a sample receiving volume and a headspace, and means for
10 withdrawing a gas sample from the headspace.

6. A method of diagnosing and/or monitoring gastric and/or lung disorders comprising:

(a) collecting a gas sample generated by a patient
15 or generated from a sample taken from the patient;
(b) passing gas from said gas sample to an array of gas sensors each having a different pattern of sensitivities to potential components of the gas sample and being adapted to provide an electrical
20 output signal in response to detection of one or more of said components; and
(c) passing said output signals to a data processing system which analyses the output signals to detect patterns indicative of the presence of predetermined
25 disorders and/or stages of predetermined disorders.

7. A method according to claim 6 which employs apparatus according to any of claim 1 to 5.

30 8. A method of claim 6 or claim 7 wherein the gas

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sample is generated in vitro from a sample of material obtained from a patient.

5 9. A method of claim 8 wherein the gas sample is generated by a process comprising treating said material sample with at least one enzyme.

10 10. A method according to claim 9 wherein said at least one enzyme comprises a lipase.

11. A method according to claim 9 or claim 10 wherein said material is treated with at least two different enzymes.

15 12. A method according to claim 11 wherein said at least two enzymes comprise a mammalian lipase and a fungal lipase.

20 13. A method according to claim 6 or claim 7 including a step of administering non-labelled urea to a patient and subsequently collecting a gas sample from the stomach.

25 14. A kit of parts for carrying out a method of any of claims 9 to 13 including apparatus according to claim 1 and at least one enzyme for use in the method of any of claims 9 to 12 or urea for use in the method of claim 13.

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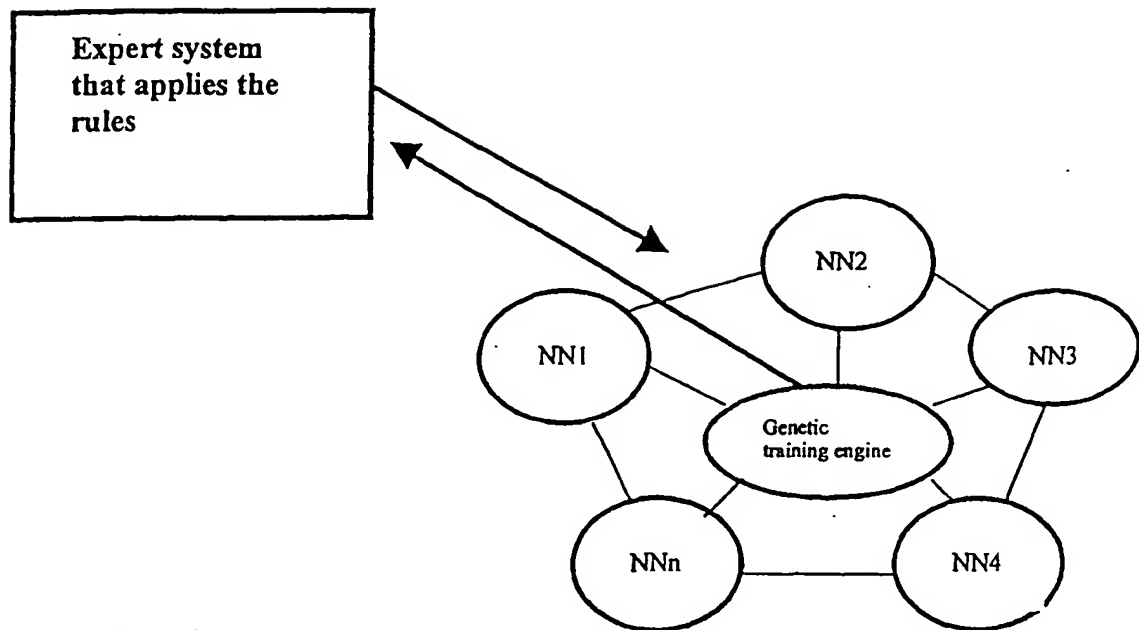


Fig 1

Data on axis 1 and axis 2 (100%)

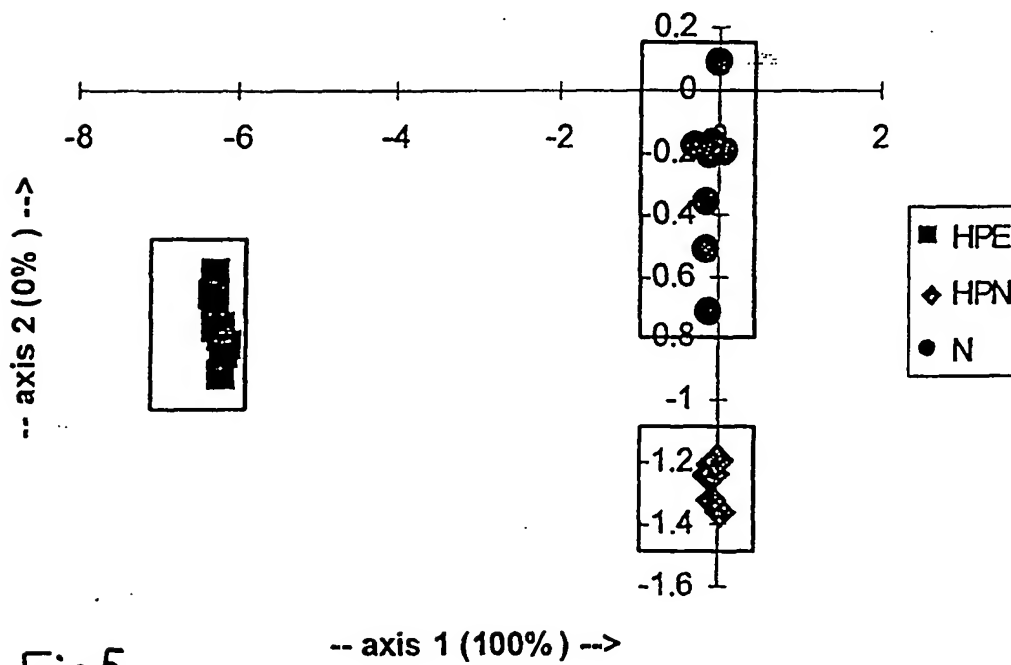


Fig5

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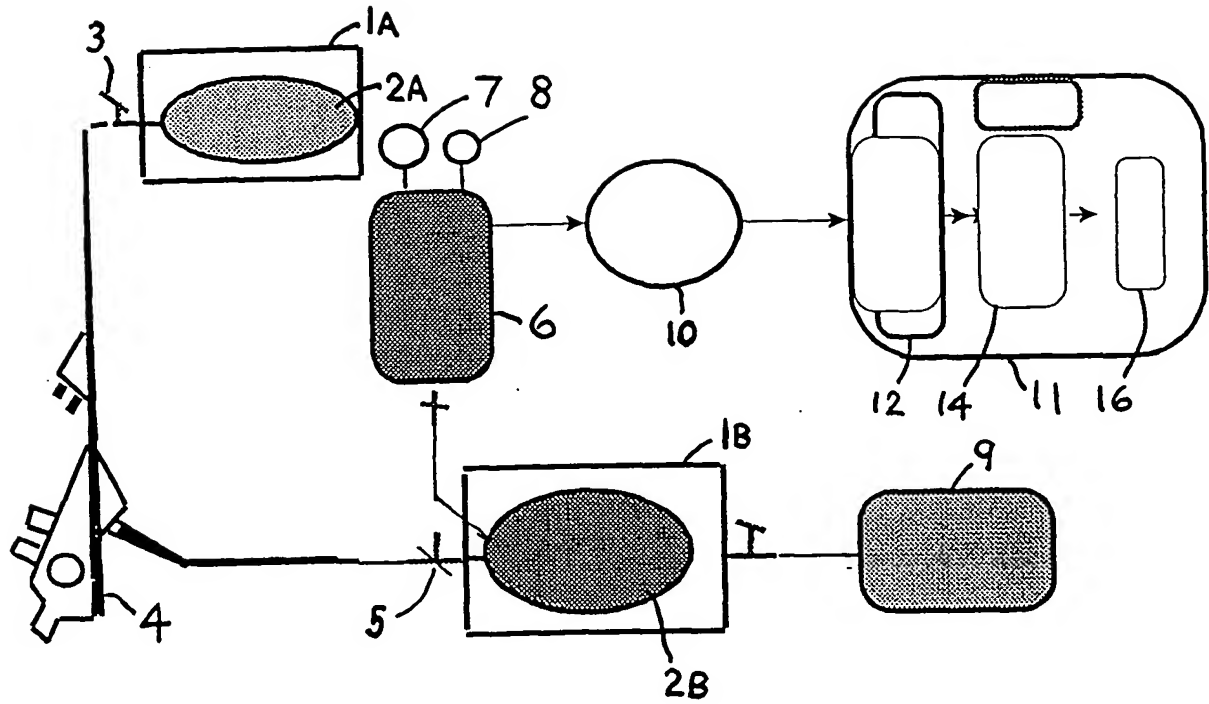


Fig 2

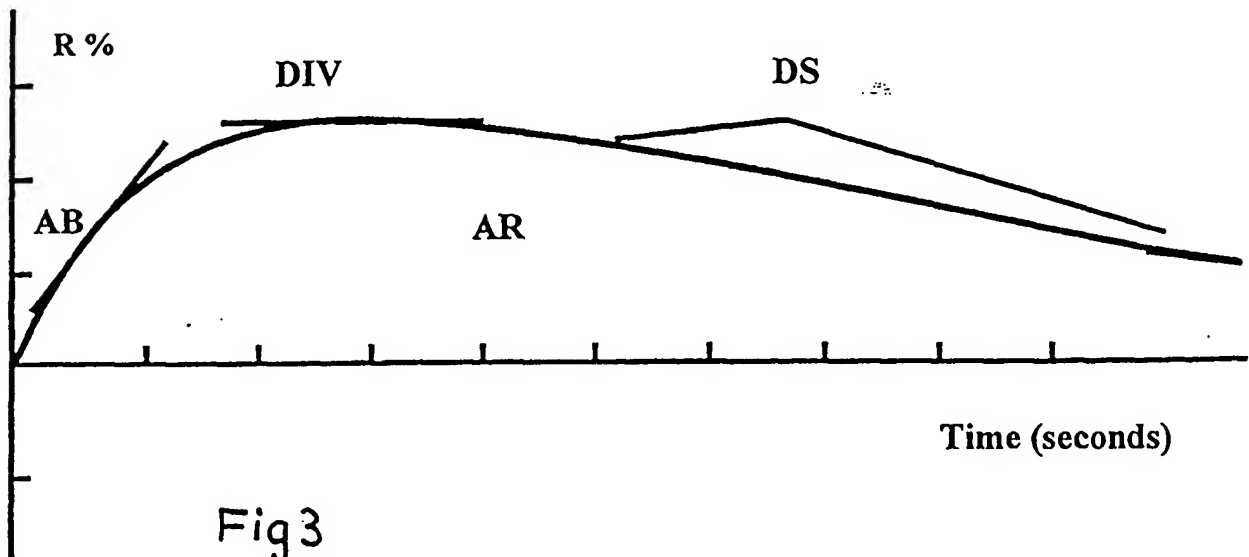


Fig3

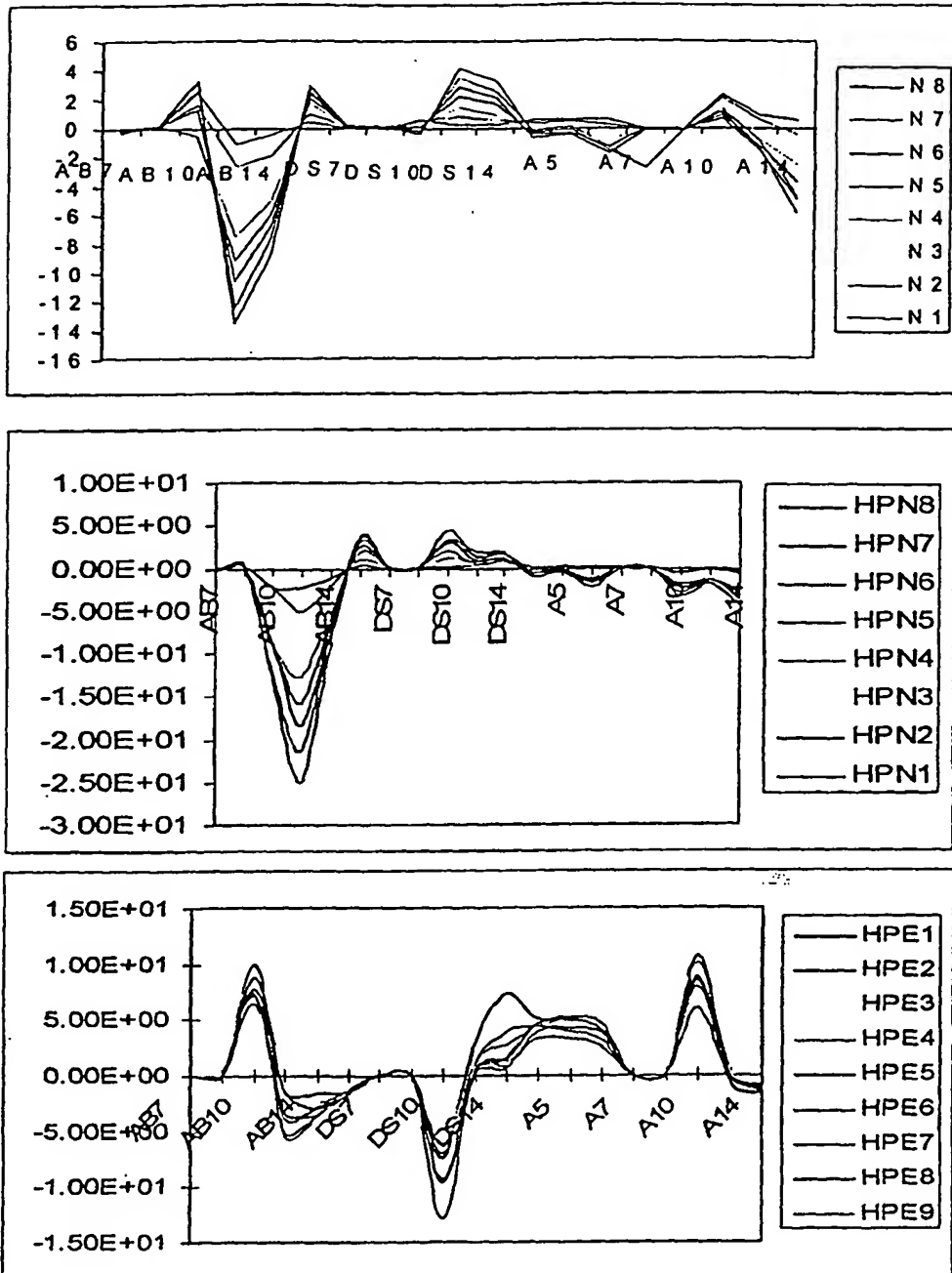


Fig 4

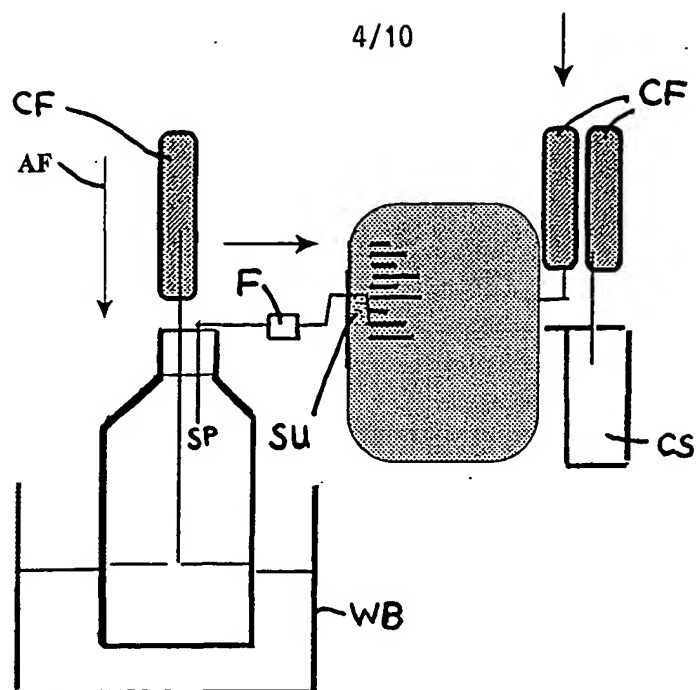


Fig 6

Data on axis 1 and axis 2 (98%)

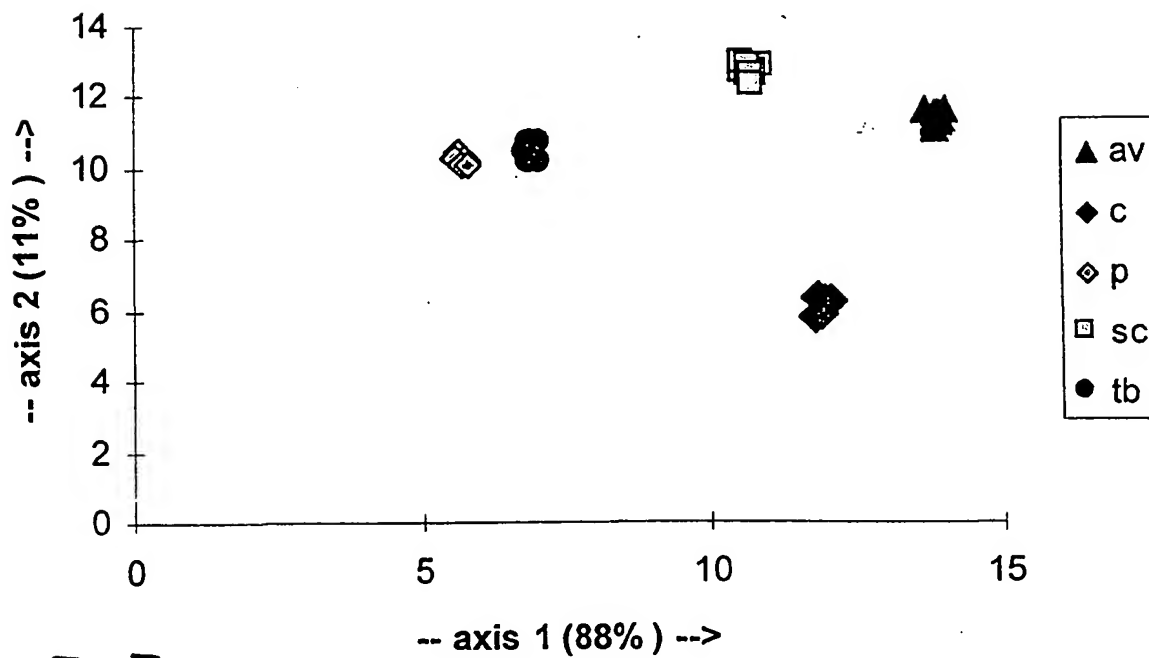


Fig 7

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Data on axis 1 and axis 2 (88%)

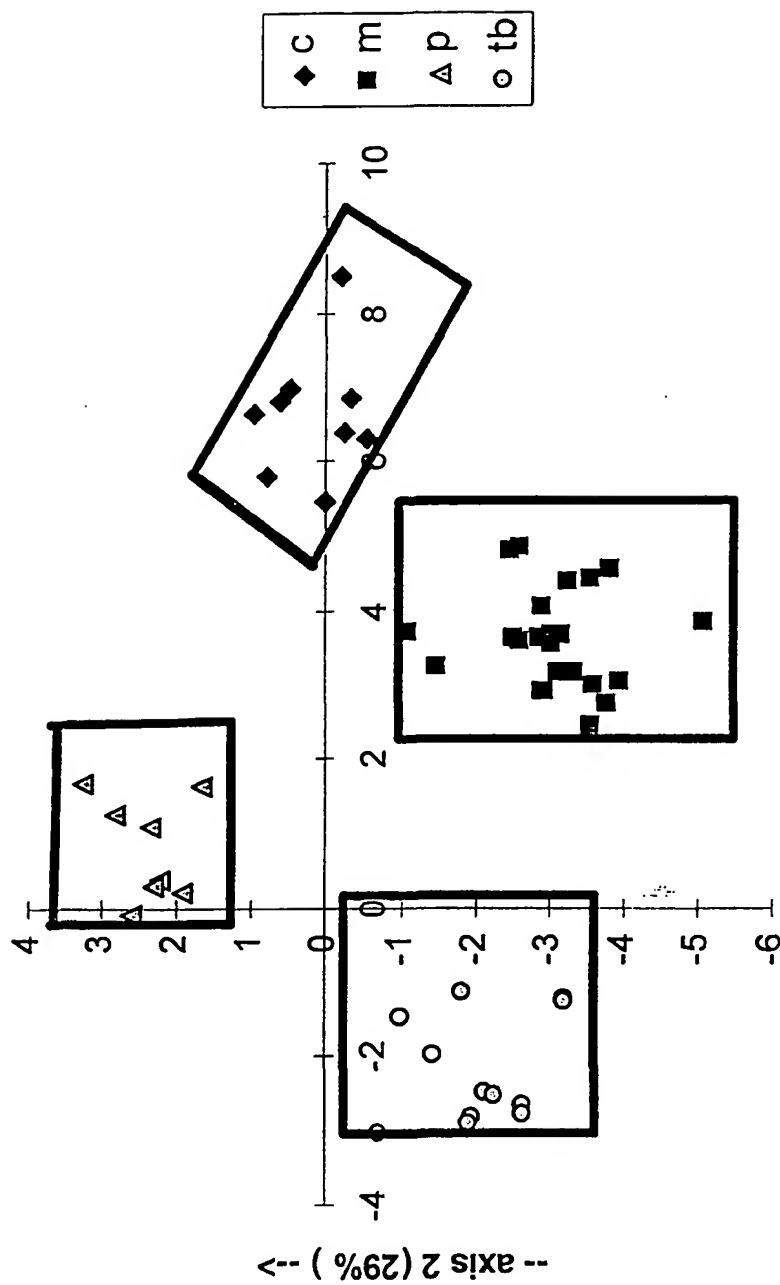
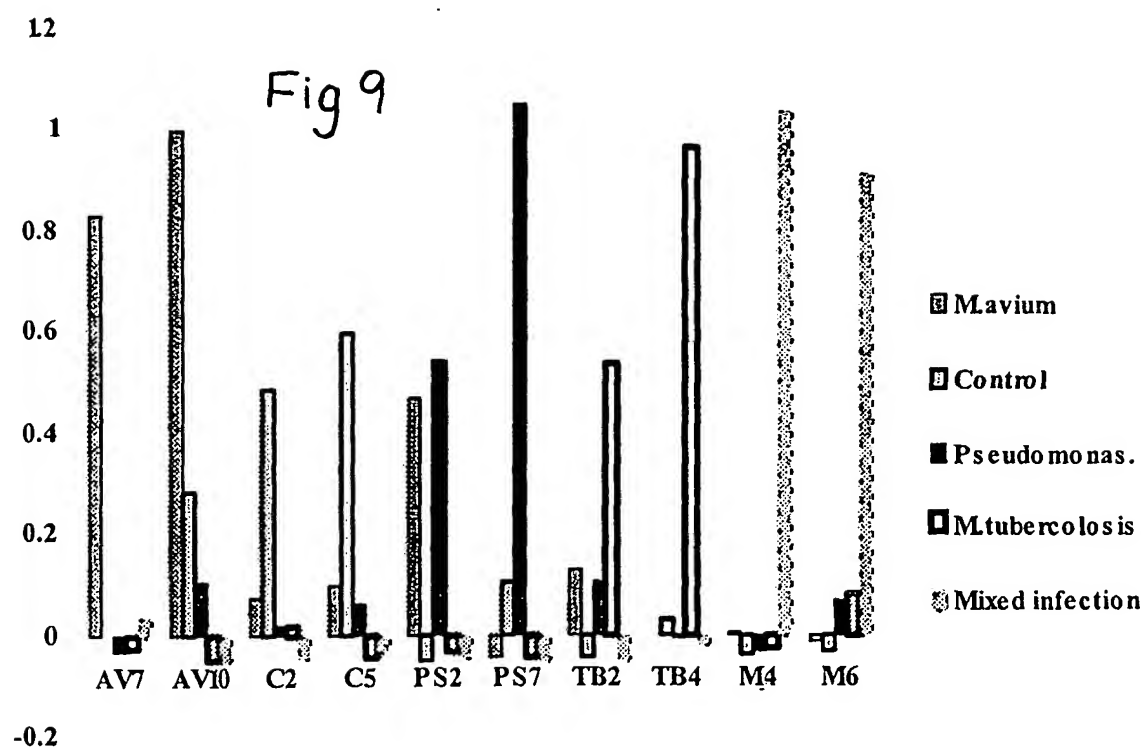


Fig 8

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Fig 10a

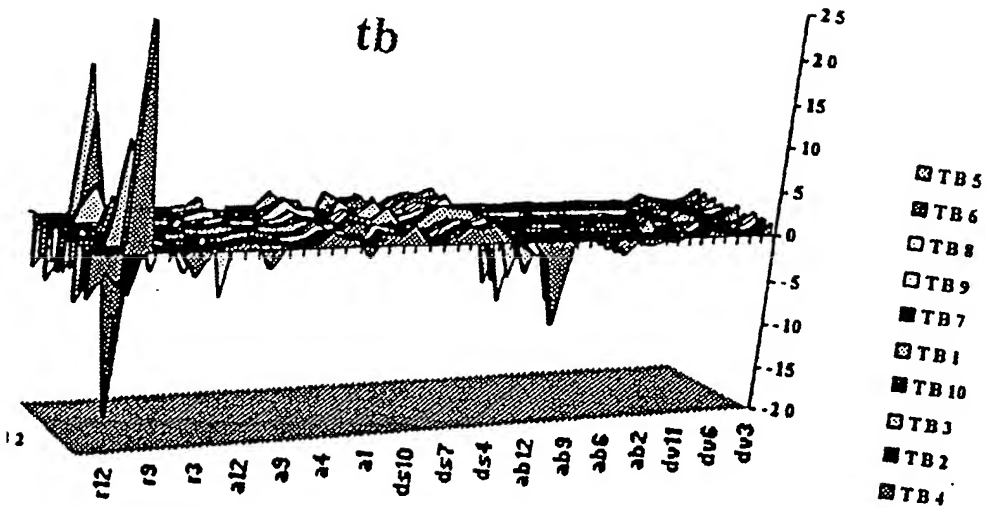
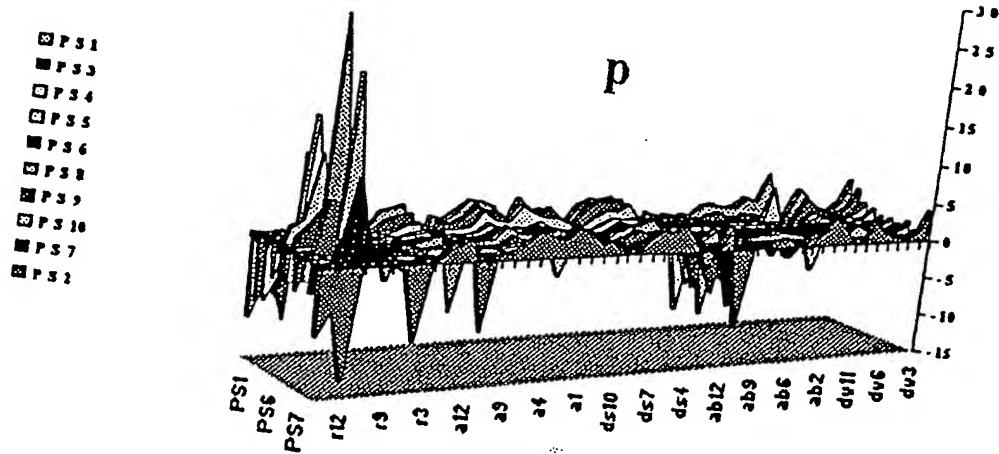
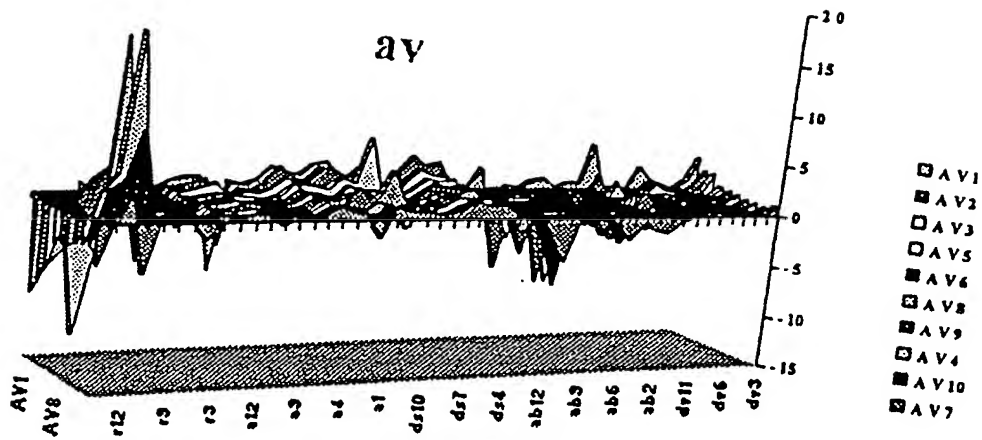
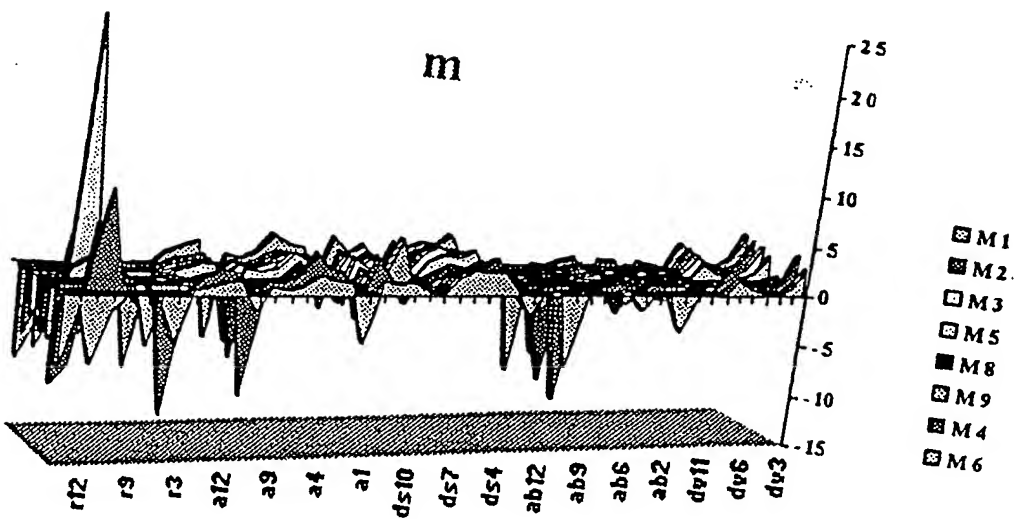
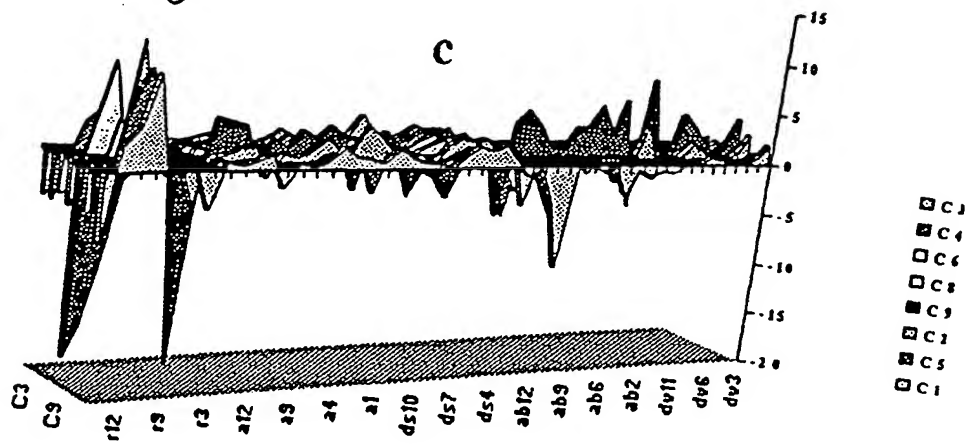


Fig 10b

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PCT/GB99/03981



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Data on axis 1 and axis 2 (83%)

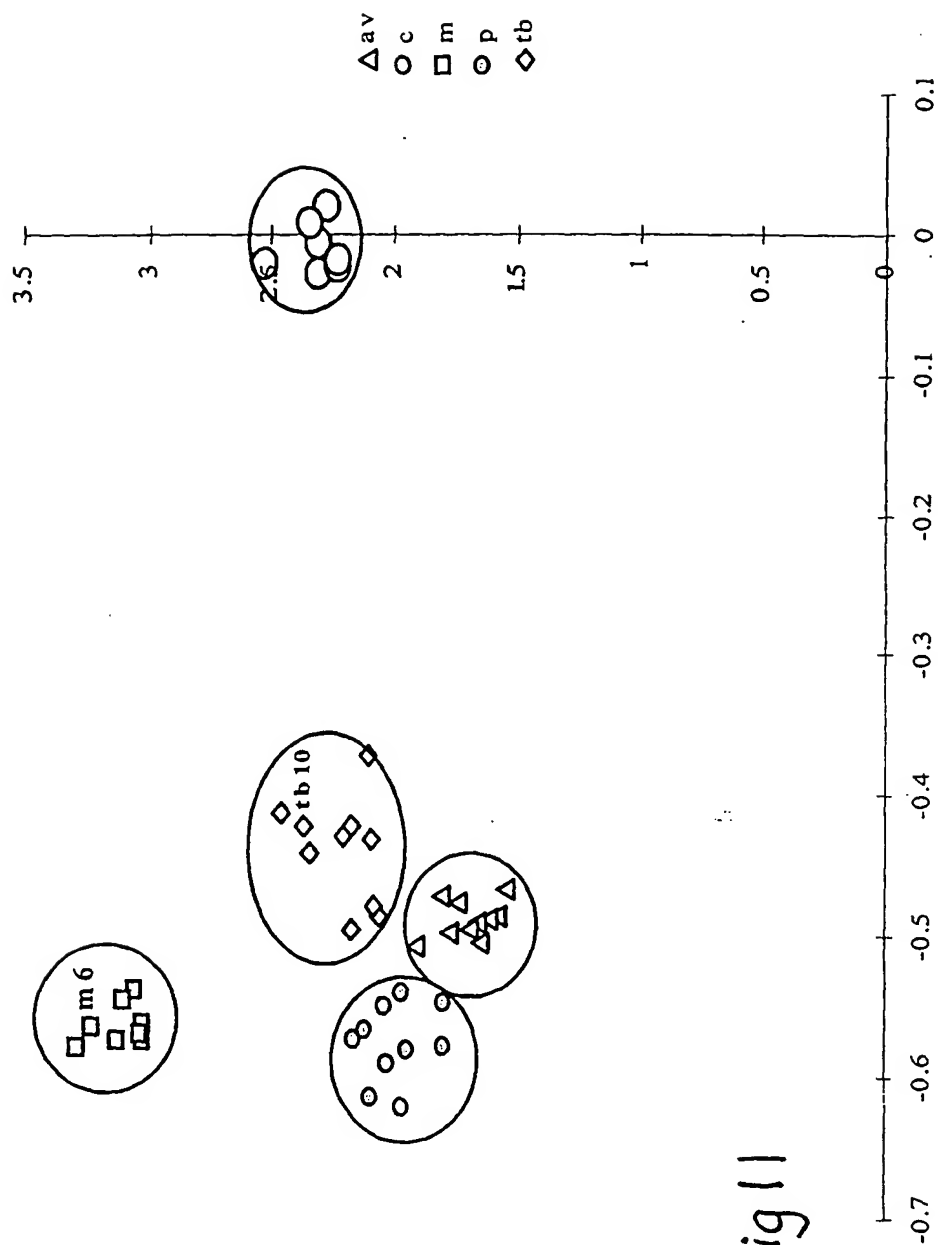


Fig 11

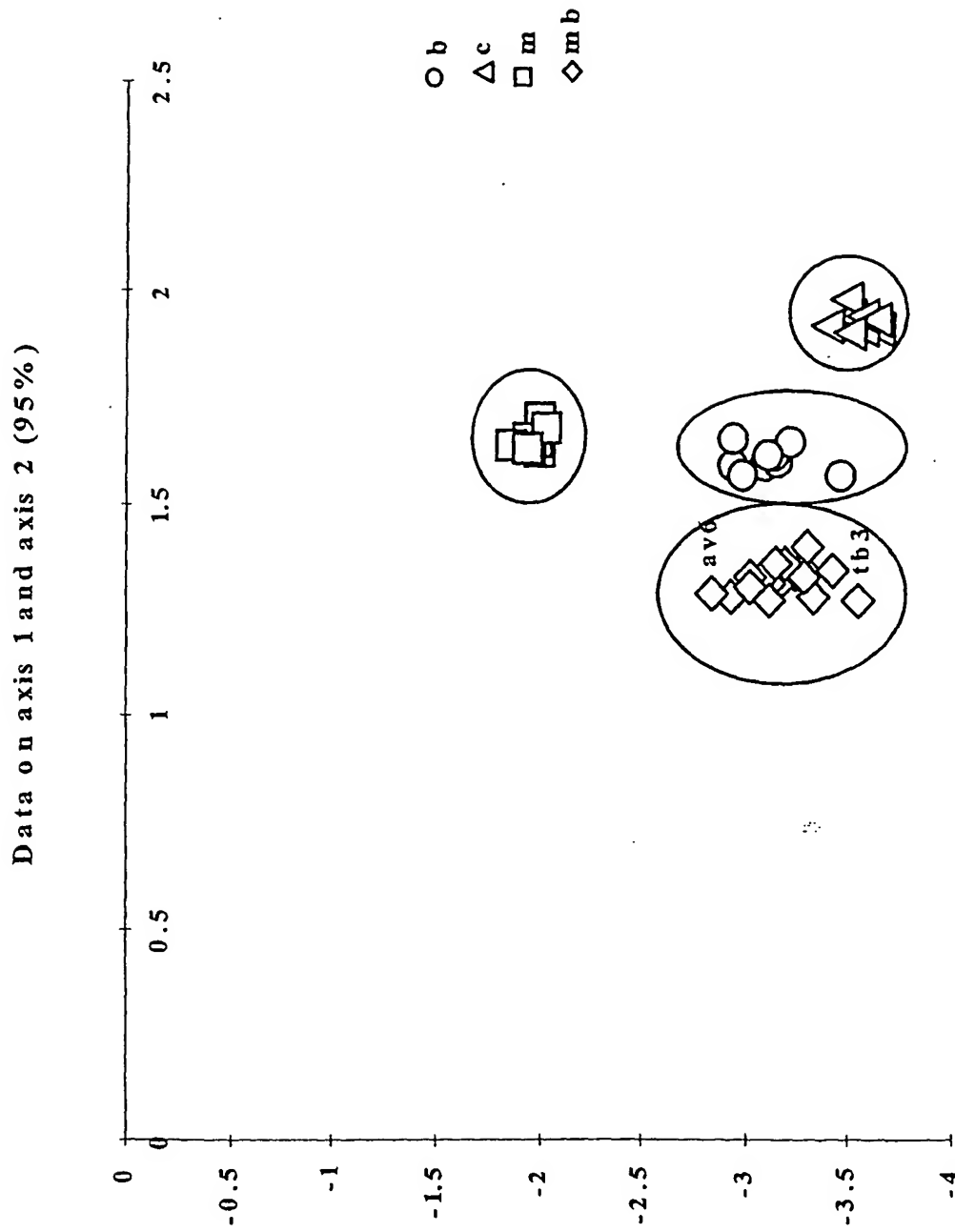


Fig 12